1926-Plat
Compensation Of Tissue-induced PSF Aberrations Using Adaptive Phase Modulation
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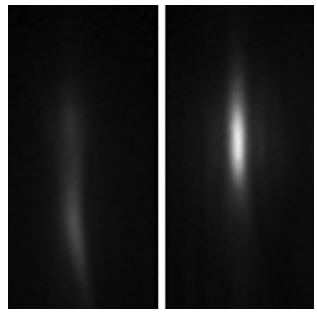

Tissue structures present index mismatches at a variety of spatial scales that can aberrate the focal volume and thus blur cellularly resolved multiphoton images acquired within biological tissues and live animals. We are surveying the types of aberrations that are caused by a variety of tissues to determine the best phase modulation strategies for adaptively correcting the excitation wavefront. A Ti:Sapphire beam is reflected off of a reflective spatial light modulator conjugate to the objective pupil plane. The excitation point-spread-function (PSF) is directly imaged with a separate objective mounted laterally to the optic axis. We find that the fluorescence signal increases with increasing size of the scattering structures. Resolution degradation, however, reaches a maximum with scatterer spatial frequencies at one tenth of the maximal frequency allowed by the focusing objective NA. PSF aberrations can be somewhat compensated by modulating the phase at the back aperture using Zernike polynomials as a basis set for increasing overall image brightness. (See figure for uncorrected vs corrected PSF's through mouse peritoneum.) Initial results show that spherical aberration is a problem, but not the only problem. (Research supported by NIH/NIBIB 41 RR04224 and NIH/NCI R01 CA116583.)

1927-Plat
Orange and Red Fluorescent Protein Optical Highlighters
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Photoconversion of fluorescent proteins (FPs) is finding increased application for routine optical highlighting in live cell imaging and supper-resolution microscopy. Photoconversion involves light-induced shifts in fluorescence properties, which are dependent on the inherent photophysical properties of the FPs. To date, all described photoconvertable FPs have been red-shifting converters. We have recently discovered that photoconversion properties are quite common among well-characterized orange and red FPs (a screen of 12 fluorescent proteins identified 8 variants exhibiting photoconversion behavior), and that several of these fluorescent proteins display a new phenomenon of blue-shifting photoconversion. A major advantage of red-to-green photoswitches is the absence of spectral bleedthrough of the initial fluorescence into the detector channel for the photoconverted species, which permits quantitative imaging of the photoconverted product as well as imaging of the initial fluorescent species using a single excitation wavelength. In addition to the blue-shifted photswitching FPs, another promising discovery from our screen is two orange FP variants that could be efficiently photoconverted to a bright and photostable far-red fluorescent species. We expect these orange optical highlighters to have significant impact for fluorescent probe development, live cell highlighting, as well as super-resolution microscopy. First, the photoconverted red species is the first fluorescent protein found to have an excitation maximum beyond 600nm. Second, photoconversion is induced using blue light, rather than near UV-light, which can greatly enhance live cell applications. Third, the red-shifted spectral properties of both the initial orange and photoconverted red species reduces the potential interference from cellular autofluorescence, and finally, these FPs are well suited for dual-probe optical highlighting applications together with photoactivatable green FPs, like PA-GFP or Dronpa.

1928-Plat
Medical Endoscopes for Multiphoton Microscopy
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Multiphoton laser scanning microscopy (MPM) is a nonlinear optical technique allowing imaging deeper into tissue while avoiding out-of-focus fluorescence and phototoxic stress on living tissues. Near infrared pulsed laser illumination nonlinearly excites the intrinsic tissue fluorescence and second harmonic generation to image the tissues. Medical-Multiphoton Microscopic-Endoscopy (M-MPM-E) can facilitate non-invasive diagnosis of diseased state in situ without resection of tissue, the grand goal of 'optical biopsy'. To enable in vivo medical applications of MPLSM, the development of compact devices is crucial. After much progress in the tabletop MPM over the past two decades, the miniature instrumentation required for endoscopy remains primitive. Here we describe the design of a small lens system suited for the endoscopy of M-MPM-E.
It has been argued previously that a microscope objective lens with high-NA and low magnification should be favorable in deep tissue MPM. We have designed and studied the properties of a reflective objective lens for M-MPM-E. Our optical design includes a modified Schwarzschild objective lens with a raster scanned laser beam, where dichroic thin film coating allows separate propagation of IR excitation and visible light fluorescence collection pathways. By engineering different magnifications for the two different spectra, it is possible to collect the fluorescence efficiently, while maintaining the high-NA for the NIR excitation spectrum. The outer diameter of the lenses of one design are 3.2mm, 0.55-NA, and the field of view is approximately $200\mu m \times 200\mu m$.
Our objective lens demonstrates close to the diffraction-limited performance. Although the central obstruction, a universal undesirable feature of reflective optics, is not avoided in our design, the effect is substantially mitigated in the two-photon point spread function. We are fabricating the device, to be included in the prototype endoscope of 5 mm maximum diameter. Research supported by NIH grant 1-R01-EB006736-02.

Platform AH: Muscle Regulations

1929-Plat
Differences in the Mechanisms of Calcium Regulation of the Acceleration of ADP Dissociation from Myosin-ADP and Myosin-ADP-Pi by Native Cardiac Thin Filaments
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We have used double mixing stopped-flow fluorescence to measure the kinetics of the dissociation of the hydrolysis products deoxymantADP (mdADP) from cardiac myosin-mdADP and cardiac myosin-mdADP-Pi by native cardiac thin filaments. Increasing the calcium concentration increases the rate of dissociation of mdADP from cardiac myosin-S1-ADP-Pi ~100 fold from 0.5 s^{-1} at pCa $>$ 7 to 50 s^{-1} at pCa $<$ 4. Increasing the calcium concentration increases the rate of dissociation of mdADP from cardiac myosin-S1-ADP-Pi only 10 fold from 15 s^{-1} at pCa $>$ 7 to 150 s^{-1} at pCa $<$ 4. These results indicate that slow dissociation of phosphate limits the rate of ADP dissociation from acto(thinfilaments)myosin-ADP-Pi and that there are different mechanisms for the calcium regulation of dissociation of the two products of myosin ATP hydrolysis, ADP and phosphate. These results support a mechanism in which the step of the hydrolysis cycle that is principally regulated by calcium is phosphate dissociation from actomyosin-ADP-Pi and do not support a mechanism such as the three state mechanism in which the regulation is a result different distributions of thin filament states in presence and absence of bound calcium that occur prior to myosin binding. This work is supported by a NIH HL84604.

1930-Plat
Effects of Elevated Solvent Viscosity on Calcium Dependence of Cardiac Myofilament Contractility
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We have previously shown with skinned skeletal muscle fibers at maximum Ca^{2+} activation and unregulated *in vitro* motility assays that solvent viscosity modulates actomyosin function in a manner consistent with diffusional limitation of a kinetic process. To determine whether viscosity influences thin filament regulatory protein dynamics, we performed experiments in cardiac muscle preparations with varying [Ca^{2+}]. First, *in vitro* motility assays were conducted using thin filaments reconstituted with recombinant human cardiac troponin and tropomyosin, and rabbit skeletal HMM and actin; solution viscosity was varied by addition of sucrose. At maximum [Ca^{2+}], we observed that thin filament sliding speed was inversely proportional to the solution viscosity. In addition, Ca^{2+}-sensitivity (pCa_{50}) of thin filament sliding speed decreased significantly with elevated viscosity ($\eta/\eta_0 \geq \sim 1.6$). For comparison with results from unloaded motility assays, single skinned porcine cardiomyocytes were used to measure steady-state isometric force and the kinetics of isometric tension redevelopment (k_{TR}) when viscosity within the myofilament lattice was elevated. Maximum Ca^{2+} activated force changed very little for sucrose $\leq$ 0.3 M ($\eta/\eta_0 \sim 1.4$) or glucose $\leq$ 0.875 M ($\eta/\eta_0 \sim 1.66$), but decreased at higher concentrations. Maximum k_{TR} decreased steeply and monotonically with increased sucrose or glucose. Ca^{2+}-sensitivity of isometric force also decreased in accord with the *in vitro* motility assay results. While either 0.3 M sucrose or 0.875 M glucose lowered k_{TR} at high [Ca^{2+}], there was little or no effect at low [Ca^{2+}]. Taken together, these results suggest that cross-bridge cycling is more affected by elevated viscosity than thin filament dynamics in cardiac muscle; changes in